Reference: *Biol. Bull.*, **151**: 182–199. (August, 1976)

NERVOUS CONTROL OF CILIARY ACTIVITY IN GASTROPOD LARVAE

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Gastropod veligers have been called "the most spectacular of all molluscan larvae" (Fretter, 1967, p. 357), displaying many adult characteristics along with an elaborate and characteristically larval structure, the velum, which serves for locomotion and food collection (Fig. 1). Light microscopic observations (Carter, 1926, 1928; Werner, 1955; Thompson, 1959; Fretter, 1967) agree on the existence of muscle and nerve fibers radiating out across the velum from the head on either side, but these accounts differ in many matters of detail. Werner (1955) and Fretter (1967) describe a network of nerve cells whose cell bodies lie in the velum, but such cells do not figure in Carter's more detailed account (1926, 1928). Carter found only the ramifying branches of nerves originating in the brain. Fretter found no nerve connections between the brain and ciliated epithelium, but such connections are depicted by Carter (1926, 1928) and by Thompson (1959). The mass of radiating nerve fibers shown by Thompson somewhat resemble the radiating fibers called retractor muscle fibers by Fretter. Here and elsewhere there may have been a failure to distinguish the two fiber types clearly. Thompson and Werner indicate that there are local, velar muscle cells, as well as muscle fibers entering the velum from the body of the larva. Allowing for differences between species, there still appear to be several important points in need of resolution.

The present study is concerned largely with elucidating the neuromuscular relationships in the velum, and with tracing the motor innervation of the ciliated cells of the preoral band, which are responsible for locomotion. Carter (1926, 1928) claimed that the ciliated cells were innervated and that the intermittent arrests of ciliary beating which he described were due to nervous inhibition. This work has been justly cited as a classic in the field of ciliary control. The pharmacology of ciliary control in veligers has been explored in some depth (Koshtoyants, Buznikov and Manukhin, 1961; Buznikov and Manukhin, 1962; Korobtsov and Sakharov, 1971) but there has been no verification of Carter's key claim regarding neurociliary junctions and in fact Aiello (1974) was unable to confirm the existence of such junctions in T. E. Thompson's electron micrographs. Neurociliary synapses have also proved elusive in bivalve gills (Paparo, 1972) where similar histological relationships might otherwise be expected to prevail.

The present study supports Carter's findings on innervation and further provides new evidence from electrophysiological recordings regarding the ciliary control mechanism. Intracellular recordings from a post-veliger pteropod larva here provide evidence complementing the results with veligers.

Taken in conjunction with the Russian pharmacological work, these results show quite clearly that the preoral cilia are strictly controlled by the larval nervous system, and that the system is probably little or no less sophisticated that the ciliary control

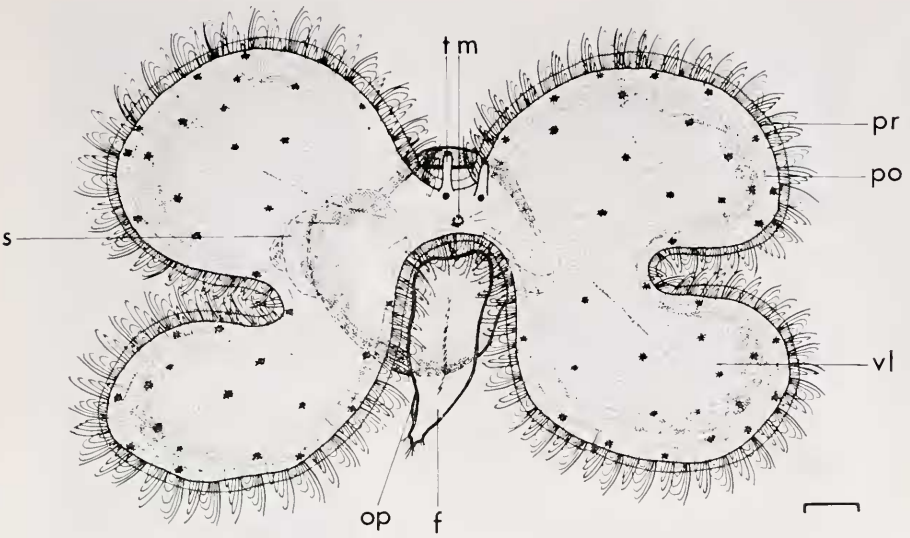

FIGURE 1. Veliger larva of *Mangelia*. Abbreviations are f, foot; m, mouth; op, operculum; po, postoral ciliated band; pr, preoral ciliated band; s, shell; t, tentacle; vl, lobe of velum; scale, 100 μ.

system in bivalve gills, currently the subject of active investigation in several laboratories (see reviews by Aiello, 1974; Jørgenson, 1975).

Materials and Methods

Several species of veliger larvae, including both prosobranch and opisthobranch genera were investigated in this study, but the reported observations refer to *Mangelia nebula* (Montagu) and to the *Mangelia* sp. termed species C (Thiriot-Quiévreux, 1969), which were convenient to study, being larger and less prone to retract into their shells than most other prosobranch species. Polytroch larvae of the gymnosome pteropod *Pneumoderma atlanticum* (Oken) were also investigated. These post-veliger larvae lack a velum, but have three ciliated rings, one at the base of the head, one in the middle of the body and a third near the posterior end (Fol, 1875). The ciliated bands persist long after the appearance of adult organs and continue to serve for locomotion even after the wings are developed.

The larvae were retrieved from freshly collected plankton hauls in the bay of Villefranche-sur-Mer during the period January to May 1975. They were isolated in clean water and used for experiments within a day or two of collection.

Fine polyethylene tubes were used as suction holders, doubling as electrodes for stimulating and recording. Tubes of about 30μ internal tip diameter were chiefly used. Attachment of these electrodes stimulates the animal initially and causes contraction and ciliary arrest, but if the suction applied is not excessive, specimens soon relax and resume normal activity. For intracellular recordings, glass microelectrodes of 40–50 megOhms resistance were used in conjunction with a Medistor A 35 electrometer amplifier, with display on a Tektronix 5102 storage oscilloscope,

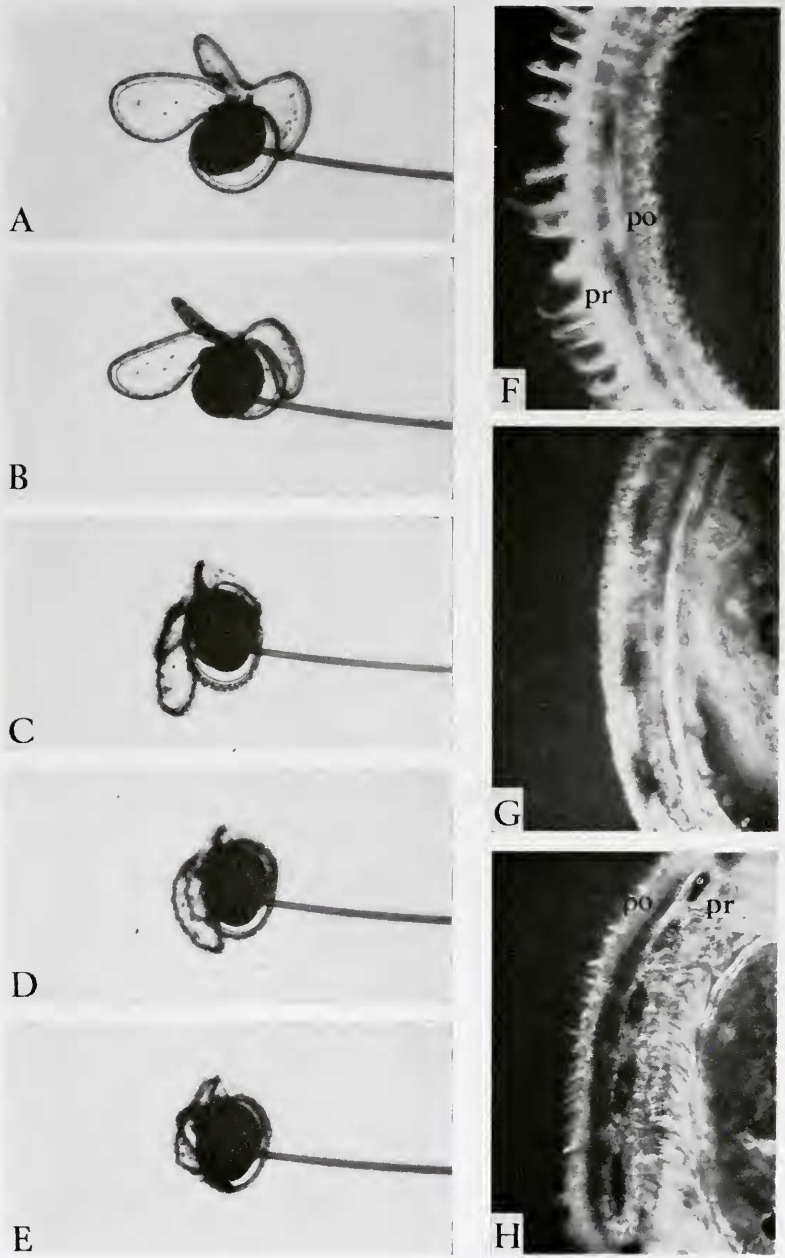

FIGURE 2. A–E, veliger attached to electrode, showing stages of recovery following a protective contraction. F–H, flash photos of velar margin showing the preoral (pr) cilia exhibiting the normal metachronal rhythm (F), arrested at start of power stroke (G) and curled inward aborally, allowing postoral (po) cilia to be seen at left hand edge(H). (See further in text.)

or a Grass 79C pen-writing oscillograph. A Grass S 44 stimulator was used for giving electrical shocks.

A Zeiss microscope equipped with bright-field, phase contrast and Nomarski interference optics was used for tracing the distribution of nerves and muscle fibers in the velum. A Zeiss Ukatron flash unit was used to photograph cilia in motion. Specimens were examined alive, either unstained or after staining with rongalit-reduced methylene blue. Portions of the velum to be examined were removed from larvae narcotised in sea water containing additional magnesium chloride to about 150 mM, sometimes with the addition of a small pinch of EGTA. Carter (1926) recommended nicotine as a good narcotic, but we have not found it to be as effective as magnesium for our purposes.

Electron microscopy was carried out on sections cut from Epon blocks of tissue fixed in 4% glutaraldehyde, postfixed in 2% osmium tetroxide, both buffered in cacodylate buffer. Uranyl acetate and lead citrate were used as stains, and sections were examined with a Philips EM 300.

Results

General observations

Veligers with the velum expanded normally (Figs. 1 and 2A) show a continuous laeoplectic metachronism in the preoral (locomotory) ciliated band (Carter, 1926; Knight-Jones, 1954; Fretter, 1967). Contact with another solid object or with the surface film causes ciliary arrest, usually along with some degree of muscular contraction, depending on the intensity of the stimulus. A sufficiently light touch causes momentary ciliary arrest with little or no muscle contraction.

In nature, ciliary arrests would result in sinking and might be evoked by a variety of tactile, and perhaps chemical, stimuli (Fretter, 1967). The folding of the velar lobes around the shell (due to contraction of the intrinsic velar musculature) or withdrawal into the shell (due to contraction of retractor fibers) would doubtless assist sinking by reducing frictional resistance and would reduce the vulnerability of the velum to damage. However, as Carter (1926) noted, these responses sometimes occur "spontaneously," *i.e.*, in the absence of a stimulus apparent to the observer. The significance of such events in relation to vertical migration will be discussed further.

The sequence of stages shown in Figure 2A–E represents recovery following a strong contraction which fell short of retraction into the shell. In E the lobes are clasped tightly around the shell; the cilia are arrested. In D, the cilia are still arrested, but the lobes are starting to relax. In C the cilia are straightening out from their recurved posture and are starting to beat irregularly. In B, metachronism is established and muscular relaxation is almost complete. The crinkling of the velar border seen in D is presumably due to the tight contraction of the marginal muscle fibers which underlie the preoral band.

Flash photos of the velar margin, viewed from the aboral side, are shown in Figure 2F-H. In F, the preoral and postoral bands are showing normal metachronism. In G, a weak stimulus has caused arrest of the cilia of the preoral band. The cilia are flexed aborally into a position corresponding to the beginning of the power stroke. A stronger stimulus (H) causes not only ciliary arrest, but a muscle

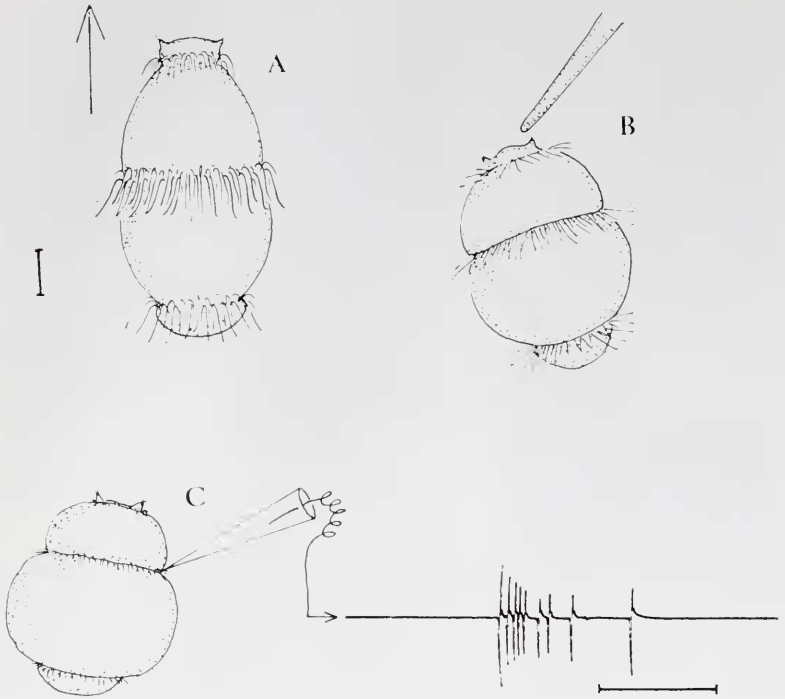

FIGURE 3. Polytroch larva of *Pneumoderma atlanticum*, swimming normally (arrow) in A, contracted with arrested cilia following brief contact with a probe (B) and more strongly contacted (C). Electrical record in C shows ciliary arrest potentials recorded extracellularly from a ciliated band. Size scale equals 100μ; time scale, 1 sec.

response: the curling aborally of the velar margin and preoral band. This brings the postoral band into view at the outer edge (left), the arrested cilia of the preoral band being seen now on their right.

We have observed no synchronized arrests in ciliated cells other than those of the preoral bands, which agrees with Thompson's (1959) findings in *Archidoris*. The cilia of the postoral bands and food groove continue normal metachronal beating when the preoral bands are arrested. Thus it is not necessary to assume, as does Richter (1973), that feeding is impossible during arrest of the locomotory cilia.

Pneumoderma larvae crawl on the bottom of their dishes or swim freely in the water by means of their ciliated bands, the cilia showing laeoplectic metachronism, like those of the veligers. Figure 3A shows a young larva extended and swimming normally in the direction shown by the arrow. A touch with a glass probe (B) causes the cilia to stop beating at the end of the power stroke or to lose their metachronism, beating weakly and irregularly. Ciliary arrest may be accompanied by some degree of muscle contraction, typically a symmetrical shortening of the whole body, which causes the sides to bulge and the cilia to be partially retracted into circular furrows. In a stronger response, such as the one shown in C, the cilia would be totally arrested and retracted until only the tips showed. These

responses are thought to be protective in nature as they result in rounding of the body, cessation of forward movement, sinking, and shielding of the cilia. Spontaneous ciliary arrests of variable duration are seen here, as in the veligers.

Older *Pneumoderma* larvae swim by the action of their cilia aided by occasional bouts of wing undulation. Stimuli which provoke ciliary arrest and body shortening also cause the wings to retract, though retraction can occur by itself, without relation to the general protective response. Other independent responses include unilateral body flexions and extrusion of the buccal mass. Cessation of the heart beat is seen during strong contractions, but whether this represents nervous inhibition or is a secondary effect of contraction, mediated perhaps by fluid pressure, has not been determined.

Isolation of ciliated bands from the central nervous system

Ciliary metachronism is quickly re-established in velar lobes cut from *Mangelia* larvae. By contrast, the distal fragments remaining attached to the head take a long time to expand and to start beating again. It is difficult to provoke ciliary arrest in pieces whose connections with the brain have been cut. Small areas may show local arrest, but there is little if any spread to adjacent regions.

Bisection of a *Pneumoderma* larva by a transverse cut just anterior to the middle ciliated band resulted in the loss of spontaneous coordinated ciliary arrests in the posterior half, and it became impossible to provoke arrests by strong tactile or electrical stimulation.

It seems that severing the connections with the brain abolishes the transmission pathway for the arrest response in both types of larvae.

Electrical correlates of ciliary arrest

Electrodes attached to the velar surface in *Mangelia* show no potential changes during normal ciliary activity, not even during the slight muscular twitches and flexions which velar lobes perform. As soon as there is a ciliary arrest, whether or not muscles contract at the same time, the electrode picks up one or a series of large (1–3 mV) potentials (Fig. 4A). Comparable events are recorded from *Pneumoderma* larvae (Fig. 3C). A single brief arrest (little more than a momentary interruption in the metachronal beating) is represented by a single potential in the electrical record (Fig. 4B, at point 1). A sustained series of potentials represents a sustained arrest, as in Figure 3C and in Figure 4B following point 2. If the interval between the potentials is long enough, as in Figure 4A toward the end of the burst, each potential can be correlated visually with a discrete postural shift in the arrested, or weakly beating, cilia. Spontaneous bursts (Fig. 4B) resemble bursts evoked by tactile or electrical stimulation.

The presumption that the potentials represent ciliary arrests rather than muscle twitches is supported by the observations that muscle twitches without arrests are not accompanied by potentials, while arrests without twitches are accompanied by these electrical signals. It is inherently unlikely that the potentials represent nerve action potentials, nerves being small, deep-lying, presumably well-insulated structures; there would be no precedent for recording their activity at the surface, even less as pulses in the millivolt range. As in echinoderm larvae (Mackie, Spencer

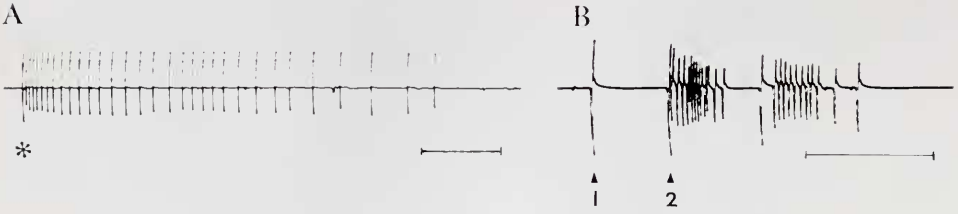

FIGURE 4. Ciliary arrest potentials recorded extracellularly from *Mangelia* (A) and *Pneumoderma* (B). In A, a 1 msec electrical stimulus close to threshold strength (asterisk) evoked a long series of potentials. In B, a light touch (1) evoked a single arrest potential, a stronger touch (2) a series. The final burst was spontaneous. Time scales equal 0.5 sec (A) and 1.0 sec (B).

and Strathmann, 1969), larvaceans (Galt and Mackie, 1971), and ascidians (Mackie, 1974; Mackie, Paul, Singla, Sleigh and Williams, 1974), it would here be proposed that the potentials accompanying ciliary arrests (reversals in larvaceans) represent the summed activity of a large number of simultaneously depolarizing ciliated cells. The resulting bioelectric currents give rise to potential changes which can be picked up all over the body surface.

Proof of the origin of the potentials from ciliated cells has been obtained by inserting microelectrodes into visually identified ciliated cells, which is feasible in young *Pneumoderma*, thanks to the large size of the cells and the feebleness of the muscular movements. As the accompanying records show (Fig. 5A, B) the events interpreted as ciliary arrest potentials in the extracellular (upper) record are revealed by the intracellular (lower) record as 50 mV depolarizations rising quickly to a peak approximately at zero potential and decaying slowly, over about 400 msecs. As shown in Figure 5B there is little summation of consecutive spikes, even when the interval between them is less than 50 msec, so each spike can be considered as an all-or-none event. The extracellular records show distortion of the wave form due to capacitance in the system. The second of two events occurring in close succession characteristically appears attenuated in the extracellular records (Fig. 5B), since the cells are already partially depolarized from the preceding potential at the time of onset of the second. The attenuation of the potentials comprising high frequency bursts, for example in Figure 4B, is explicable on the same basis.

TABLE I

Comparison of metazoan ciliary arrest potentials, measured intracellularly.

	Resting potential (millivolts)	Amplitude (millivolts)	Rise time (milliseconds)	Decay time (milliseconds)
Corella[1]	35–40	45–50	40	1500
Mytilus[2]	60	<20	30	170
Pneumoderma[3]	50	50	15	400

[1] Mackie *et al.* (1974).
[2] Murakami and Takahashi (1975).
[3] Present paper.

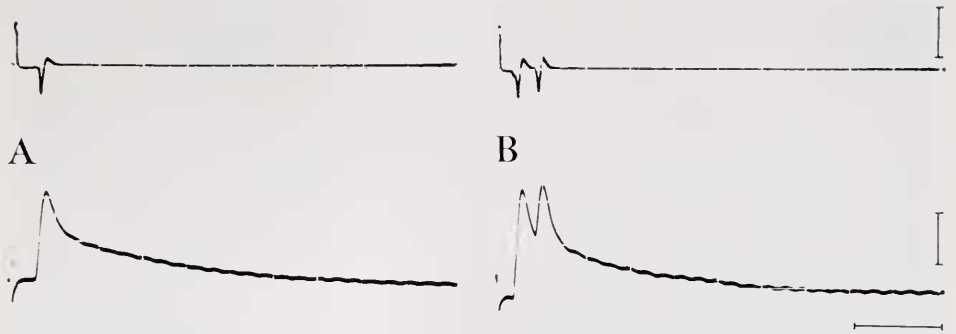

FIGURE 5. *Pneumoderma:* simultaneous extracellular (upper) and intracellular (lower) records of ciliary arrest potentials. A shows a single arrest potential, B two such potentials 45 msec apart. Time scale equals 100 msec; amplitude scales, 3 mV (extracellular) and 20 mV (intracellular).

The duration of ciliary arrest probably corresponds fairly closely to the duration of depolarization of the cells, but this relationship has not been accurately determined. To do so would require simultaneous monitoring of the ciliary beating with a photomultiplier, a technique used for the first time in a metazoan by Murakami and Takahashi (1975).

During normal ciliary activity the membrane potential is steady and unwavering as in the ascidian *Corella* (Mackie *et al.*, 1974) and in *Mytilus* (Murakami and Takahashi, 1975).

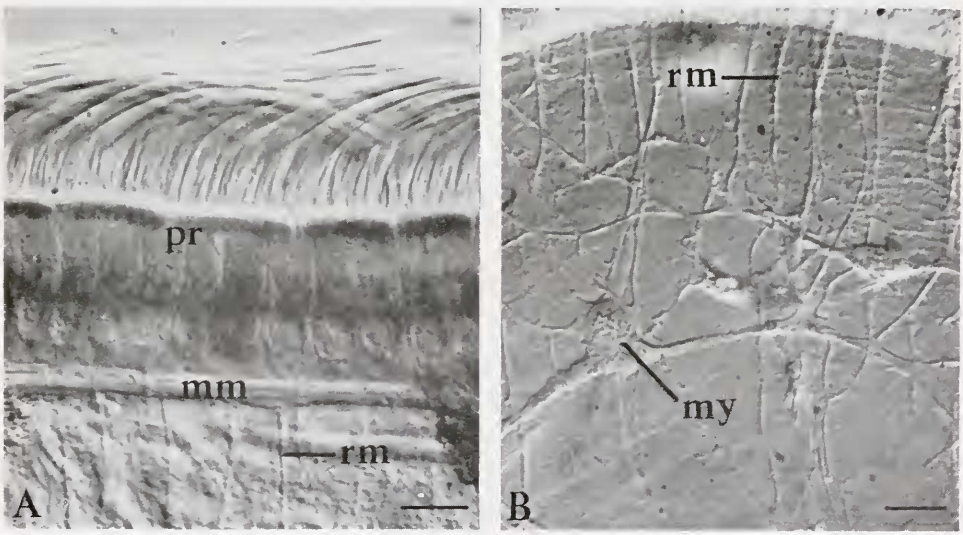

FIGURE 6. *Mangelia:* interference contrast photographs of the living velum. A., velar margin showing preoral band, B., area adjacent to margin. Abbreviations are mm, marginal muscle band; my, myocyte; pr, ciliated cells; rm, radial muscle strand; scales, 20 μ.

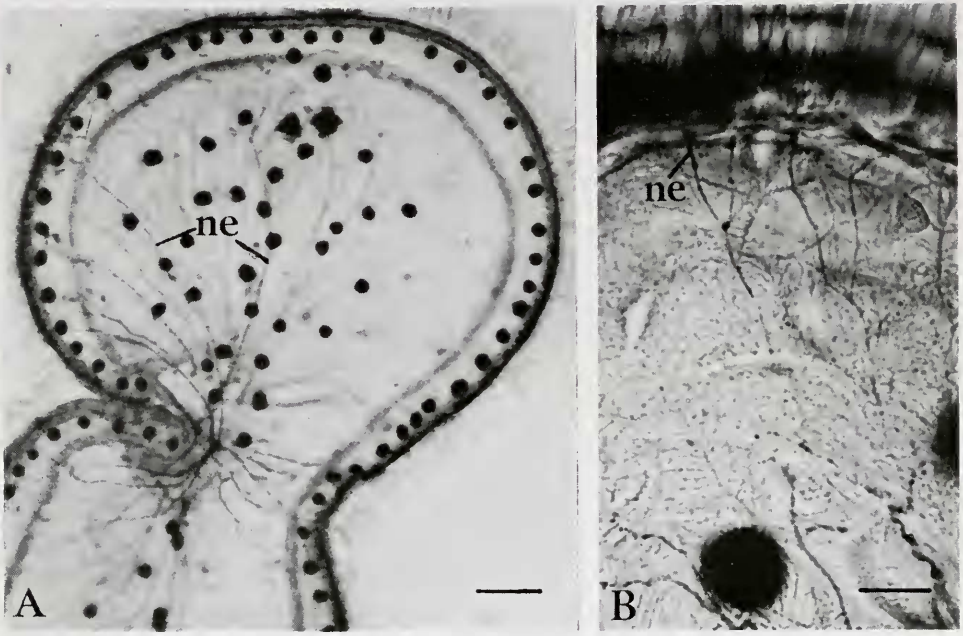

FIGURE 7. *Mangelia:* velum stained with methylene blue: A., whole lobe; B., an enlarged area near the margin. ne represents nerve process; scales, 50 μ in A, 10 μ in B.

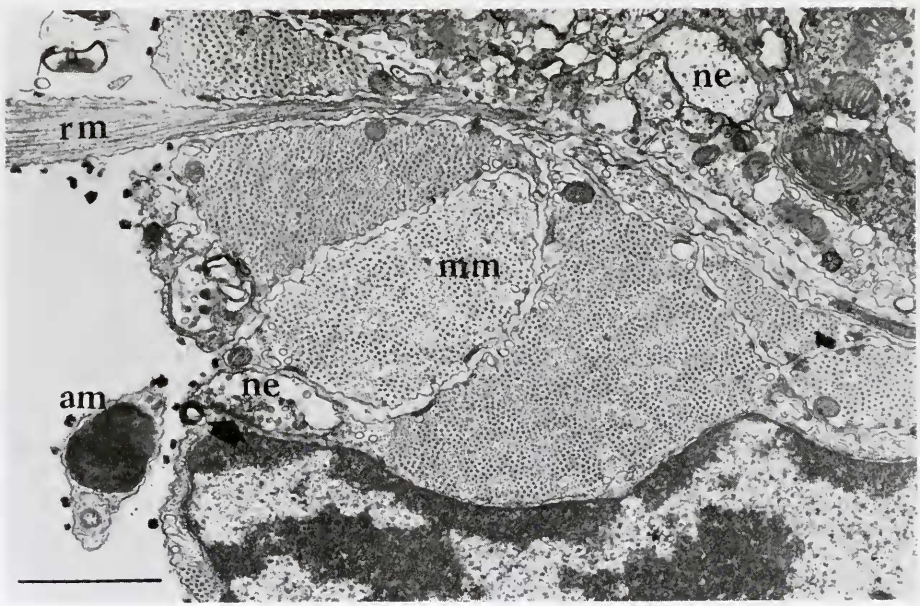

FIGURE 8. *Mangelia:* section of velar margin in the region of the marginal muscle band (see Fig. 10). Abbreviations are am, process of amoebocyte; ne, nerve process; mm, marginal muscle fiber; rm, radial muscle fiber. Arrow shows a neuromuscular junction; scale, 1.0 μ.

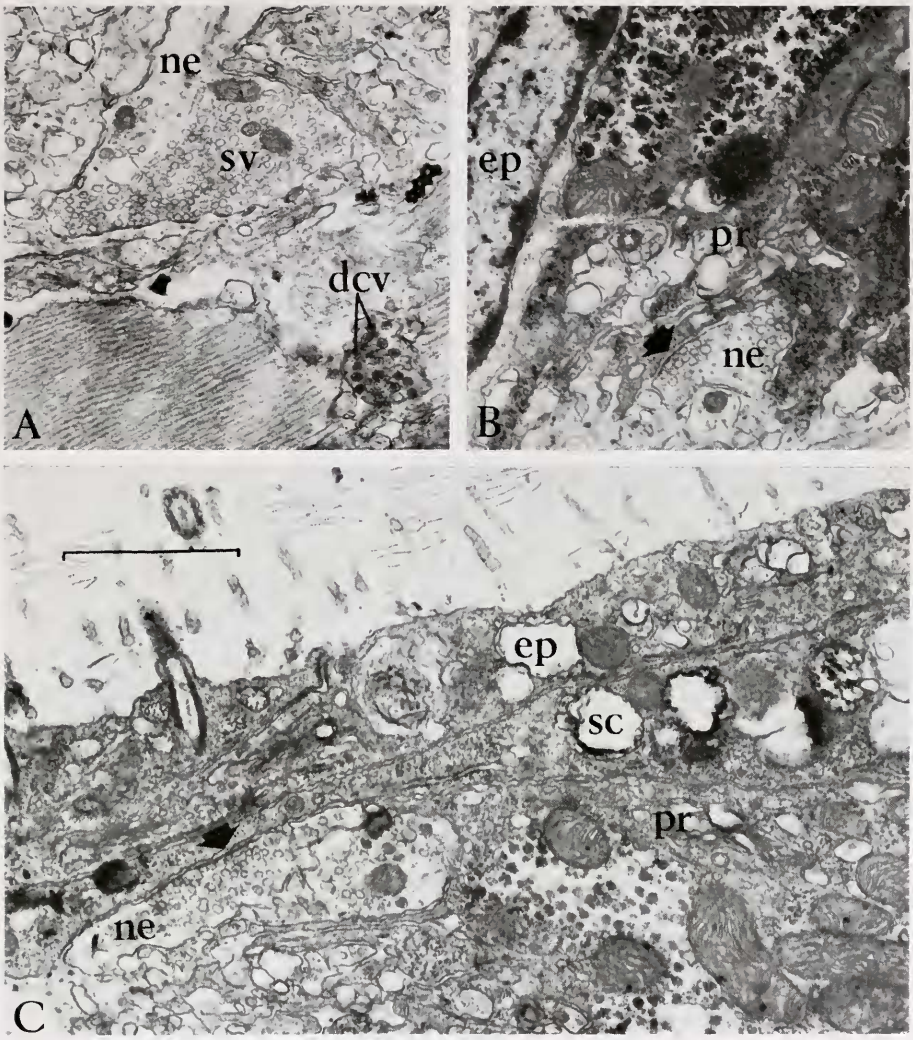

FIGURE 9. *Mangelia:* sections of velar margin in region of marginal muscle band showing two sorts of nerve vesicles (A), ciliated cell of preoral band (B) and supporting cell (C) (see Fig. 10). Abbreviations are dcv, nerve process with dense-cored vesicles; ep, epithelial cell; ne, nerve process; pr, ciliated cell; sc, supporting cell; sv, small clear vesicles. Synapses (arrows) are characterized by thickened presynaptic membranes and aggregations of small clear vesicles. Scale equals 1.0 μ.

Morphology and fine structure

The following account refers chiefly to *Mangelia nebula.*

Preoral band. The appearance of the living cells under the interference microscope is shown in Figure 6A. Further details of the ciliated cells as seen by light microscopy are given by Carter (1926, 1928) and Fretter (1967). The

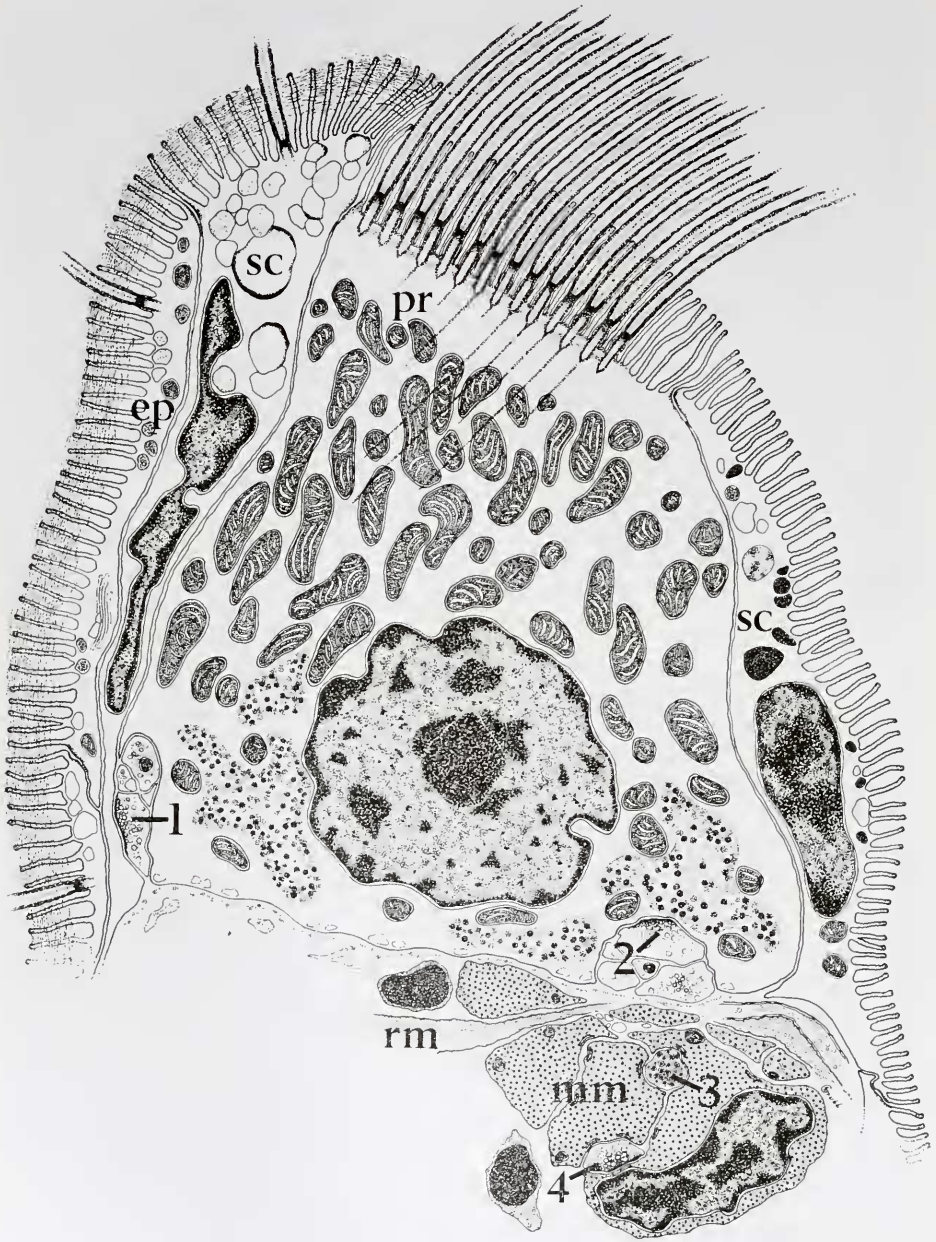

FIGURE 10. *Mangelia:* drawing summarizing histological relationships seen in radial sections through the preoral ciliated band. The ciliated cell (pr) contains large numbers of mitochondria and glycogen islets. Supporting cells (sc) lie on either side. Epithelial cells (ep) cover the velum, abutting on the supporting cells. All these cells bear microvilli. On the left, which is the side of the "food groove" (Fretter, 1967), the microvilli are embedded in a fibrillar mesh. The marginal muscle bundle (mm) lies toward the aboral side, with radial muscle fibers

major features of the cells as revealed by electron microscopy are summarized in Figure 10 and need little additional description. The cells are distinguished by a complex ciliary apparatus, by large numbers of mitochondria, and by deposits of granular material, resembling the pools of glycogen rosettes (α-glycogen) seen in *Helix* (McGee-Russell, 1968) and many other molluscs (Fig. 9 B, C). They receive synapses from local neurites which run in bundles within folds of the basal membrane (Fig. 9B). They are flanked on oral and aboral sides by supporting cells which are themselves partially clad in epithelial cell processes. Both the latter cell types are ciliated, but the cilia are short and far apart rather than being long and bunched together into compound units like those of the locomotory ciliated cells. All three cell types bear microvilli. On the food groove side (left in Fig. 10) a felt-like mass of layered fibrils is interspersed among the microvilli (Fig. 9C).

The supporting cells contain granules of amorphous, electron-dense material of unknown composition. They are innervated (Fig. 9C), which suggests that release of the product may be under nervous control. The epithelial cells also contain secretion bodies, perhaps mucus, but receive no synapses. All three cell types are joined by septate desmosomes near their outer edges.

Muscle layout. In addition to the velar retractor muscle system (Fretter, 1967, 1972), there is a local muscle system, corresponding to Fretter's "intrinsic muscles," consisting of the processes of myocytes which lie wholly within the velum. This "muscle net" consists of branching myocytes whose processes intermingle with the retractor fibers; the net lies on both sides of the velum and is specialized into a thick band, the marginal muscle, which runs circularly around the edge beneath the preoral band, toward the aboral side (Figs. 6A and 8). Contraction of this band probably causes the aboral inflexion and crinkling of the velar edge noted earlier as a response to stimulation. Radially arranged fibers deriving both from the retractor system and from myocytes in the muscle net run up to and around the marginal muscle. Their role would be to control general posture and curvature of the velum and to fold it when it is withdrawn.

The distribution of myocytes comprising the intrinsic musculature of the velum is highly organized and consistent within a given species, but varies widely between different species. Part of this muscle net is shown in Figure 6B, from the aboral side of the velum. The cells are readily distinguishable from amoebocytes. The latter have shorter processes and often lie completely separated from other amoebocytes. Their cell bodies show a very irregular and variable appearance and are generally less compact than those of myocytes. They are found in the blood spaces as well as near the surface epithelia. The myocytes might be confused with multipolar neurons, but their processes are more numerous, thicker, and less delicate than typical nerve net neurites and more prone to adhere together. Their cell bodies are less regular and compact, and no part of the cell shows an affinity for methylene blue in preparations where nerve processes of central origin are well stained. On the positive side, electron microscopy shows nucleated cell profiles with processes

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(rm) passing out toward oral and aboral surfaces. Nerve elements are numbered as follows: 1, process synapsing with a supporting cell; 2, process synapsing with ciliated cell; 3, process containing dense-cored vesicles; and 4, process synapsing with a myocyte.

containing muscle filaments in the regions where myocytes are seen by light microscopy.

Under the electron microscope (Fig. 8) the muscle fibers, both in the local net and in the retractor strands, are seen to be highly differentiated structures with thick and thin filaments and small flattened sub-sarcolemmal cisternae. They are often seen to be associated with processes of amoebocytes. Neuromuscular synapses are frequently seen in the marginal muscle band.

Innervation. In the intact, living velum stained with methylene blue, nerves can be seen running from the point of attachment beside the head out across the velum in all directions (Fig. 7A). The prominent dark structures in this figure are pigment cells, which do not appear to be innervated. Only rarely are results sufficiently good to allow the fine terminal nerve branches to be selectively stained; but here and there this happens, and nerve terminals can be followed right into the marginal muscle band and to the bases of the ciliated cells (Fig. 7B). No evidence was found for a general velar nerve net (i.e. interconnected processes of cells with cell bodies dispersed across the velum), although there is a small group of local neurons on the aboral side near the head on either side.

While we agree with Carter's account on most points, we have only occasionally seen nerve endings running up between the ciliated cells. Generally they go no further than the bases of these cells. Under the electron microscope, neurites were found mingling with muscle fibers and enveloped in pockets within the bases of the ciliated cells. The innervation is rich and synapses are abundant.

Two sorts of vesicles are seen in the neurites, small clear vesicles with diameters ranging between 335 and 560 Å, and larger dense cored vesicles with diameters 560 to 835 Å (Fig. 9A). The two sorts are usually well segregated into different neurites.

Synapses are recognized by the usual criteria for gastropods (Amoroso, Baxter, Chiquoine and Nisbet, 1964), *viz*, thickened junctional membranes and massed, small, clear vesicles. Dense cored vesicles are rarely found at synapses.

As already noted, synapses are made with three different cell types: muscle cells, ciliated cells and supporting cells. The epithelial cells are not innervated. No nerve endings identifiable as sensory terminations have been spotted. The ciliated cells of the postoral (feeding) band do not appear to be innervated, although nerve bundles lie near their bases. This point needs further study and verification.

All the foregoing remarks in this section have referred to *Mangelia* veligers. The fine structure of *Pneumoderma* larvae has not been examined in as much detail, but it can be stated that the locomotory ciliated cells in this species are also richly innervated, receiving synapses whose appearance under the electron microscope closely resembles that of the synapses in *Mangelia*.

Discussion

The evidence presented here confirms the findings of Carter (1926, 1928) regarding the existence of nerve fibers running from the brain to the velar preoral ciliated band. We cannot yet say how many ciliated cells are supplied by branches of the same nerve fiber, but there is clearly a very rich innervation; and in all probability each cell receives at least one nerve ending. There is no need therefore

to assume extensive spread of excitation within large populations of ciliated cells, as in ascidian stigmata (Mackie *et al.*, 1974), although limited local spread might still occur. Motokawa and Satir (1975) report the slowly spreading arrest of ciliary beating in *Mytilus* following localized damage by laser irradiation, confirming earlier work on *Elliptio*. These spreading arrests are probably electrotonically mediated by current flow through gap junctions between the ciliated cells.

Nudibranch veligers gain the ability to arrest their cilia only after the innervation is established (Buznikov and Manukhin, 1962). It is shown here that separating the ciliated epithelium from the brain permanently abolishes coordinated ciliary arrests. In the ascidian *Corella* by contrast isolated bits of gill continue to exhibit arrests, showing that a conduction system and pacemakers are developed on the local level (Mackie *et al.*, 1974). Contrary to some earlier reports, we have found no evidence for a local nerve net in the velum, but only the nerve fibers which come from the brain.

At the fine structural level we have been able consistently to show neurociliary synapses both in veligers and gymnosome larvae. These appear to be the first neurociliary synapses described for a mollusc. Paparo (1972) has found a rich innervation in bivalve gills but no synapses. In annelid trochophore larvae, neurociliary and neuromuscular synapses similar to those described here are reported (Holborow, 1971). In the trochophore of *Phyllodoce*, cholinesterase activity has been demonstrated histochemically along the inner surfaces of the ectoderm cells, especially those of the prototroch, by Dr. T. Lacalli, University of British Columbia (personal communication). The veliger synapses structurally resemble interneural synapses in terrestrial gastropods (Amoroso *et al.*, 1964), having a dense population of small synaptic vesicles. All the evidence points to these junctions as the propable mediators of ciliary arrest, but the chemical transmitter concerned is not known. Acetylcholine occurs in gastropod nervous systems (Kerkut and Cottrell, 1963) and is suspected to be the interneural transmitter at junctions having this type of morphology in bivalves (Myers, 1974), but proof is lacking. Buznikov and Manukhin (1962 and earlier reports cited) isolated a substance from veliger larvae which inhibits ciliary beating and decreases sensitivity to the excitatory action of serotonin. They suggest that this substance may be the normal mediator of ciliary arrests. However, it is not acetylcholine, and despite intensive investigation its identity remains unknown. A further argument against acetylcholine as the transmitter comes from Carter's (1926) observation that curare has little or no effect on veligers.

Our electron microscope work has demonstrated the existence of a certain number of velar neurites stuffed wtih dense-cored vesicles. Similar vesicles occur in many other molluscs (Gerschenfeld, 1973). In gastropods, a variety of evidence implicates them as a site of serotonin (Taxi and Gautron, 1969; Cottrell and Osborne, 1970; Jourdan and Nicaise, 1970). In bivalves, on the other hand, they are more likely to contain dopamine (Myers, 1974 and authors cited).

It is well established that serotonin (5-hydroxytryptamine) stimulates the beating of velar cilia in opisthobranch veligers (Koshtoyants *et al.*, 1961; Buznikov and Manukhin, 1962; Korobtsov and Sakharov, 1971) as it does in isolated bivalve gill preparations (Aiello, 1957, 1960; Gosselin, 1961). Jørgenson (1975), who reviews more recent work in this field, found that serotonin did not affect the rate

at which intact mussels cleared suspensions of yeast cells. He suggests that the serotoninergic innervation serves only to *maintain* (rather than to accelerate) the activity of the lateral cilia in the intact animal. The mechanism of action of serotonin is uncertain, but it is still effective on veligers when all ions other than magnesium are removed from the external medium (Korobtsov and Sakharov, 1971) which makes it unlikely that the drug acts by changing membrane permeability according to these authors. Paparo and Murphy (1975a and b) give evidence that it works by mobilizing calcium stored intracellularly.

Thus evidence from several sources points to a dual ciliary control mechanism in veligers. Both involve the nervous system, but only one (the arrest system) is likely to be associated with neurociliary synaptic transmission. The other perhaps works by releasing serotonin in tissue spaces near the ciliated epithelium.

It is too early to say how closely this picture corresponds to the situation in bivalves. The apparent absence of neurociliary synapses in bivalves is puzzling since the arrest response is so similar to what we see in veligers. In both cases arrest appears to be dependent on external calcium ions (Korobtsov and Sakharov, 1971; Satir, 1975), and indeed an influx of Ca^{2+} is evidently the critical event in ciliary arrest or reversal generally (see reviews by Naitoh and Eckert, 1974; Aiello, 1974).

Our electrical recordings show that in the gymnosome larvae, and to all appearances in veligers, ciliary arrest is associated with a depolarization lasting some 400 msec. These events (Table I) are much larger than comparable events recently recorded in *Mytilus* by Murakami and Takahashi (1975) and more closely resemble ciliary arrest potentials in tunicates (Mackie *et al.,* 1974). We envisage the depolarizations being initiated by inward synaptic currents and being accompanied by a temporary increase in calcium conductance. Thus they would be excitatory rather than inhibitory events in the usual neurophysiological sense, although the effector response is spoken of in terms of inhibition. Repetitive firing results in sustained depolarization and prolonged arrest. We would like to correlate the electrical and mechanical events by the precise technique exploited by Murakami and Takahashi (1975).

Until more is known about the life of these larvae in the sea, not much can be said about the functional utility of ciliary control, although it seems obvious that any animal which relies so heavily on ciliary effectors would find numerous advantages in being able to regulate their activity. It is reasonable to suppose that ciliary arrests, along with the muscular retraction of the velum which often accompanies them, would serve as a means of protection and escape from damaging stimuli (Fretter, 1967). The veligers of *Nassarius obsoletus* stop swimming and settle on the bottom in response to a chemical factor emanating from suitable substrates (Scheltema, 1961), but it is not clear if this response involves ciliary arrests of the kind we are dealing with here.

With regard to "spontaneous" arrests, G. Richter's studies raise some interesting possibilities. This author (Richter, 1973; Richter and Thorson, 1975) notes that swimming veligers normally swim upward, because the distribution of lighter and heavier parts is such as to cause the velum to face upward. Upward locomotion takes place at a velocity of 36–52 m/hr in the laboratory, which would permit in theory (as appears likely in practice) as ascent of 200 m during the diurnal migra-

tion. The velocity of sinking in nonswimming larvae was measured in the laboratory at 72 m/hr. However data for the migration cycle in the sea give a descent rate of only 10 m/hr which is taken to indicate that descending larvae sink in fits and starts, presumably by alternately arresting the cilia for brief periods and then letting them beat again. Thus, spontaneous ciliary arrests may serve a functional role in regulating the rate of sinking during downward migration. It would be worth testing the effects of light on the frequency of ciliary beating since light is an important stimulus for many migrating species (Vinogradov, 1970).

The experimental work was carried out during a visit by G. O. Mackie to France under the NRC-CNRS scientific exchange program, and was further assisted by travel and operating funds from NRC. We thank P. Bougis, Director of the Station Zoologique, for providing the necessary facilities. The electron microscopy was done at the University of Victoria on an instrument granted by the National Research Council of Canada.

Summary

1. The locomotory cilia of *Mangelia* and *Pneumoderma* larvae undergo arrests spontaneously and in response to tactile stimulation. These events are often associated with muscular contractions in an overall response thought to be protective in nature.

2. Isolation of the ciliated bands from the central nervous system abolishes the ability for coordinated ciliary arrests and the cilia show continuous metachronal beating.

3. Recordings with suction electrodes attached to the surface show patterns of electrical signals during periods of ciliary arrest. Intracellular recordings with glass microelectrodes from single ciliated cells in *Pneumoderma* show rapidly rising, slowly decaying, all or none 50 mV spikes when the cilia undergo arrest. There are no fluctuations in membrane potential during metachronal beating.

4. The existence of a rich motor innervation supplying the ciliated epithelium in *Mangelia* has been established using optical and electron microscopy. The nerve endings appear to derive from neurons whose cell bodies are located in or near the central nervous system. The evidence for a local system of neurons forming a nerve net, as described by some authors, is not supported by the present work.

5. Under the electron microscope, neurociliary synapses have been identified. Each ciliated cell in the preoral band of *Mangelia* probably receives at least one synapse. These junctions presumably mediate the arrest response. Synapses are characterized by small, clear presynaptic vesicles in the range 335–560 Å. Similar junctions are made with muscle cells and with supporting (presumed secretory) cells which lie adjacent to the ciliated cells.

6. Neurites containing dense-cored vesicles (560–835 Å) are found near the ciliated cells, but such vesicles are rarely found at synapses and never predominate in them. Taken in conjunction with findings from other gastropods, this observation appears to complement existing pharmacological evidence for an excitatory role for serotonin in molluscan veligers. Comparisons with the dual system of ciliary control found in lamellibranch gills are suggested.

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